

Defining the Roles of Various Lysines and Arginines in Amot Lipid Binding

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One of the defining traits of cancerous cells is proliferation. The focus of this study is on the proliferation of mammary cells. As an adaptor protein, the Amot membrane binding event is key to the localization and sorting of proteins responsible for cellular differentiation, proliferation, and migration. The Amot coiled-coil homology domain (ACCH) is a lipid-binding domain responsible for cholesterol affinity and binding to endothelial membranes. Our working hypothesis is that the ability to modulate Amot lipid-binding will lead to means to prevent ductal cell hyperplasia progression into breast cancer tumors. We will determine which residues are responsible for lipid-binding by changing positively charged lysine and arginine into uncharged or negatively charged amino acids. Approximately 40 of these mutations have been screened using a liposome binding assay which mimics how the protein binds with the cell membrane by using an in vitro mixture of lipids similar to that seen in endothelial cells. Forster resonance energy transfer (FRET) was used to confirm significant decreases in lipid binding of ACCH mutants selected from the liposome binding assay, as energy transfer only occurs when the tyrosines in the protein and the Dansylated liposome are in close proximity to each other. In order to saturate the binding affinity of the mutants, the liposomes will be combined with cholesterol in increasing amounts. It has been found that Amot protein is concentrated in areas of PI with higher levels of cholesterol. This will provide a target for the ACCH domain to associate with in the membrane. Mutants deemed important from this study will then be transformed into human cells to study their effects on cell polarity, signal transduction, cell shape, and cellular proliferation.

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